Vasodilator Responses to Moderate Hypoxia after Submaximal Adenosine Injection or Coronary Occlusion in Isolated Perfused Guinea Pig Hearts

DAVID F. STOWE

With the technical assistance of Kevin M. Cumming

SUMMARY My objective was to observe if coronary vasodilator responses after bolus (0.2 ml) intracoronary injection of adenosine (1-5000 μM) or after coronary artery occlusion (5-30 seconds) are modified over a range from mild to severe hypoxia [outflow oxygen tension (PO2V) = 119 ± 9 to 10 ± 2 (mean ± SEM) torr]. The vasculature of ten isolated, paced (240 beats/min), nonworking guinea pig hearts was perfused by the Langendorff technique with a fortified Krebs-Ringer solution at 37.5°C and at a constant pressure of 55 torr. Graded hypoxia was produced by randomly altering perfusate FO2 to one of five predetermined levels. Individually, maximal hypoxia, maximal adenosine injection, and 30-second occlusion increased vascular conductance by 98, 100, and 98%, respectively. At constant myocardial oxygen consumption (MVO2), PO2V was inversely proportional to baseline conductance (r = 0.42, P < 0.01); during normoxia (PO2v = 119 torr) both log-dose adenosine concentration and occlusion period were directly proportional to peak conductance (r = 0.78, P < 0.01; r = 0.73, P < 0.01). Low doses of adenosine or short occlusions significantly shifted the conductance intercept to higher values but did not change individual PO2v vs. conductance slopes. Similar results were found in the relationship between PO2v and overflow volume after adenosine injection or occlusion. Moreover, both the excess volume flow with low doses of adenosine and the volume of reactive hyperemia were unaffected by PO2v when MVO2 was unchanged. As hypoxia became more severe and MVO2 decreased, reactive hyperemia decreased and the flow debt increased. This study indicates that adenosine or coronary occlusion produces additive, noninteractive coronary vasodilation in the presence of tissue hypoxia. One possible explanation for these results is that as conductance rises, the common receptors for endogenous and exogenous adenosine become increasingly saturated.


BOTH cardiac hypoxia and release of coronary occlusion elicit profound coronary vasodilation (Hilton and Eichholtz, 1925). The metabolic hypothesis proposes that the metabolic activity of the heart is related closely to the coronary blood flow (Berne, 1964; Haddy and Scott, 1968). Under conditions of hypoxia and coronary occlusion, the decrease in the supply of oxygen is considered critical for initiation of events leading to vasodilation. Dilatation may be produced by a severe drop in tissue oxygen tension per se (Gellai et al., 1973) but more likely results from change in myocardial metabolism arising from a reduction of oxygen delivery (Olsson, 1975). It has been demonstrated that tissue concentrations of various vasoactive chemicals rise when oxygen supply is decreased (e.g., with arterial hypoxemia or occlusion) or when myocardial oxygen consumption (MVO2) is increased (e.g., with increased cardiac work). During perfusion of the coronary vasculature with fluid deficient in oxygen, adenosine is released into the venous drainage; there is a rise in the tissue levels of adenosine together with a drop in effluent oxygen tension (PO2) and an increase in coronary flow (Rubio et al., 1974; Schrader et al., 1976; Schrader et al., 1977a). Following transient coronary occlusion, adenosine appears in the effluent, and tissue levels of adenosine rise together with an increase in flow (Rubio et al., 1969; Olsson, 1970; Rubio et al., 1974; Schrader et al., 1977a; Olsson et al., 1978). Effluent PO2, however, rises above control during reactive hyperemia (Coffman and Gregg, 1961; Olsson and Gregg, 1965; Olsson, 1975; Ruiter et al., 1978). With infusions of catecholamines to increase cardiac work, adenosine is released as MVO2 and flow rise and coronary sinus PO2 decreases (Wiedmeier and Spell, 1977).

Because adenosine is a potent vasodilator when injected into the coronary arteries (Drury and Szent-Györgyi, 1929) and because it may play a significant role in mediating coronary vasodilation during hypoxia (Berne, 1963) and following occlusion (Rubio et al., 1969), I undertook this study to...
observe if the dilator responses to exogenous adenosine and to coronary occlusion are modified during dilution induced by arterial hypoxia.

**Methods**

Ten albino English short-haired guinea pigs (350-550 g) of either sex were stunned by a blow to the head. Within 2 minutes, thoracotomy and pericardotomy were performed and the ascending aorta was isolated and cannulated distal to the aortic valves. The hearts were perfused immediately at constant pressure (55 torr) by the Langendorff technique with a freshly prepared, modified Krebs-Ringer bicarbonate solution (in mM: NaCl, 118; KCl, 4.7; MgCl₂ × 6H₂O, 1.1; CaCl₂ × 2H₂O, 2.0; KH₂PO₄, 1.1; NaHCO₃, 8.0; d-glucose, 5.6; sodium pyruvate, 2.0; d-mannitol, 16.4). Sodium pyruvate has been shown to improve heart function with ischemia (Bünger et al., 1975), and mannitol was added to reduce extravascular accumulation of fluid. The perfusate (osmolality, 315 mosmol/kg) was filtered (20 μm; Millipore Corp.) immediately before use. Immediately after aortic cannulation, the pulmonary artery was cut, the left atrium was opened, and the mitral valve was cut. Next, a cannula was inserted into the right ventricle through the pulmonary artery (PE 200; Intramedic), and the pulmonary veins and vena cava were ligated. These procedures assured continued closure of the aortic valve so that the heart performed no external work. The perfusion system, made entirely of glass and nonmetallic tubing and connectors, was jacketed in a thermostatically controlled water circulation system (Haake E52) to maintain a constant temperature (37.5°C). Hearts were submerged to the aortic root in a bath of the perfusate solution. Coronary sinus effluent collected from the pulmonary artery cannula was allowed to drain at the level of the right atrium (pressure = 0) except when 1-ml samples were collected anaerobically during steady-state flow for gas and pH analysis (Acid-Base Analyzer System; Radiometer Corp.). Inflow gas tensions and pH were measured from a port in the tubing near the heart. A series of gas flowmeters (rotometer tubes 600-603; Matheson Gas Co.), connected in a parallel fashion to individual cylinders of O₂, CO₂, and N₂, were used to mix and selectively vary the fractions of these gases equilibrating the perfusate. Lateral aortic (coronary) pressure (Gould-Statham P-23 ID) as well as phasic and mean aortic (coronary) flow (Biotronix BL 610-2A with a BLC 2024 Series 2000-C extracorporeal probe) were monitored continuously on a polygraph (Grass Instruments; model 7). “Zero” flow was checked frequently by temporarily diverting coronary flow around the probe. Periodic calibration against direct volume/time measurement showed flows from 1-20 ml/min to be both linear and reproducible. The area under mean flow curves was measured with an integrator (Grass Instruments; 7P10) or by planimetry. Flow was expressed as ml/ min per g wet heart weight; vascular conductance was calculated as mean inflow rate divided by aortic (coronary) pressure. MVO₂ was calculated as the mean coronary inflow (ml/min per g) times the inflow-outflow PO₂ difference (torr) times O₂ solubility in saline at 38°C (2.82 × 10⁻³ ml O₂/ml per torr).

**Protocol**

The flow and heart rate were allowed to stabilize for about 20 minutes; hearts which did not exhibit a spontaneous regular heart rate of over 200 beats/min and at least a doubling of diastolic flow with a 0.2 ml bolus of 5000 μM adenosine or a 30-second occlusion were discarded. Tightening the ligature around the vena caval-right atrial junction usually reduced heart rate to <200 beats/min. The hearts then were paced at 250 beats/min (40 Hz; 20 msec; 0.1-2 V) to prevent asystole with bolus injection of higher doses of adenosine (2000-5000 μM) and to eliminate variations in heart rate since heart rate affects diastolic flow.

Hearts were perfused initially using a control (normoxic) gas mixture. For these experiments “normoxia” is defined as the gas mixture (about 95% O₂, 5% CO₂) which gave the highest inflow PO₂ at a pH of 7.42. The three levels of “moderate hypoxia” were produced by reducing the fraction of O₂ in the gas mixture (pH 7.42) without decreasing MVO₂. MVO₂ dropped with the two levels of “severe hypoxia.” In random order, inflow PO₂ was reduced to one of five predetermined levels by adjusting rotometer gas flow in such a way that inflow pH and carbon dioxide tension (PCO₂) remained unaltered. Coronary sinus PO₂ data were grouped according to the selected gas fraction. Each heart was exposed to at least three of the randomly selected oxygen levels for up to about 15 minutes with a return of normoxia after no more than two hypoxic levels. Only near the end of each experiment was each heart exposed to the maximal hypoxic level. During steady-state flow at each level of inflow oxygen, I rapidly injected in a 0.2-ml bolus of 1, 2, 20, 200, 2000, or 5000 μM adenosine (Sigma Chemical Co.) in saline into a side-arm port to observe peak flow responses. The volume of the cannula between the site of injection and the aortic root was about 0.5 ml. The injections were randomized; if a higher dose preceded a lower dose, saline first was injected to flush the port. Also in random order, I occluded aortic inflow for 5, 15, or 30 seconds at each oxygen level. Excess flow following adenosine injection and reactive hyperemic flow following occlusion were quantified both as peak diastolic flow and as the integrated volume in excess of mean baseline flow. Baseline flow is the steady-state flow during different levels of hypoxia or during normoxia. For these experiments, volume flow debt (or excess) is defined as the occlusion deficit (ml/g) minus the volume over-flow (ml/g) during reactive hyperemia.
Data Analysis

Each heart served as its own control both for levels of inflow hypoxia and for adenosine injections and occlusion time. There was no difference in either precontrol or postcontrol flows with injections of adenosine or with occlusion, nor was there any change in baseline flow, once established, at each level of hypoxia. Following no more than two levels of hypoxia, a control period (normoxia) was inserted. Because no difference in these control flows (P < 0.01, paired t-test) was found, the control flows were pooled so that there were 20 data points for the normoxic control period. The data, grouped according to the level of hypoxia (rotometer setting), adenosine dose, and occlusion time, were analyzed using single and multiple analyses of variance and multiple linear regression with case combinations (BMD O3R program) on an IBM 7040 computer. Individual linear regression data were compared by the Simultaneous Test Procedure for homogeneity of slopes (Sokal and Rohlf, 1969). Only data at constant MV02 were subjected to these analyses.

Results

A portion of a typical experiment is shown in Figure 1. During normoxia [inflow oxygen tension (PO2a) = 577 torr], both bolus injections of increasing concentrations of adenosine and increases in occlusion duration caused corresponding increases in peak diastolic and mean flows as well as volume overflow. Mild hypoxia (PO2v = 275 torr) by itself increased baseline flow. Peak flow with adenosine injections or following occlusions was higher during mild hypoxia than during normoxia, but the differences between peak flows and the steady-state flows produced by mild hypoxia alone were either less or unchanged. Similarly, the tracing shows that the volume overflow in excess of the baseline flow following adenosine or occlusion was either less or unchanged during the period of mild hypoxia.

Effect of Hypoxia Alone on Coronary Flow and MV02

Table 1 shows that as inflow oxygen (PO2a) was reduced by adjusting the fraction of gases in the perfusate to a random, predetermined level mean coronary flow increased and oxygen outflow (PO2v) decreased; PCO2a and pHa, however, were kept nearly constant (P > 0.10). Mean pH, at each level of oxygenation remained constant (7.31 ± 0.1) except at the lowest level (PO2v = 10 ± 2 torr) when it dropped to 7.28 ± 0.1 (P < 0.01). pHa, PCO2a, pHv, and PCO2v did not change when linearly regressed on PO2v. MV02 did not change with a drop in PO2v between 119 ± 9 and 33 ± 3 torr; however, below this latter value, MV02 fell precipitously (P < 0.001) due to severe hypoxia. The percentage of O2 extraction rose (P < 0.05) when PO2a fell below 461 ± 10 torr but fell again with severe hypoxia (PO2v = 10 ± 2 torr).

Effect of Adenosine Plus Hypoxia

Figure 2 summarizes the effects of reduced PO2a and of increasing doses of adenosine on peak diastolic coronary flow. Steady-state diastolic flow rose by 62% (4.2 ± 0.8 to 6.8 ± 1.2 ml/min per g) from normoxia (PO2v = 119 ± 9 torr) to moderate hypoxia (PO2v = 33 ± 3 torr); MV02 did not change. Flow increased by 91% (8.0 ± 0.6 ml/min per g) with severe hypoxia (PO2v = 10 ± 2 torr) (not shown). Adenosine (5000 µM) produced a maximum (100%) rise in peak diastolic flow (4.2 ± 0.8 to 8.4 ± 0.5 ml/min per g) during normoxia. As the log dose of adenosine was increased during normoxia, flow increased linearly (r = +0.76; P < 0.01). Although not significant, there was a tendency for the highest dose of adenosine (5000 µM), which produced a maximum peak flow during normoxia, to elicit an even greater maximal peak flow with increasing levels of mild hypoxia.

In Figure 3, I have displayed the data with PO2v as the independent variable and have calculated peak vascular conductance as the dependent variable. In addition the vascular responses to extreme hypoxia (PO2v = 10 ± 2 torr) are included. Conductance was found to be inversely proportional to PO2v between 119 and 33 torr (r = -0.39; P < 0.01), and peak conductance was found to be directly proportional to the log-dose adenosine concentration (r = +0.78; P < 0.01). However, at successively higher adenosine concentrations, the slopes of the linearized conductance vs. PO2v relationship flattened. Between the PO2v range of 119 to 33 torr, the slopes for 1, 2, and 20 µM adenosine were statistically equal to the control slope (P < 0.01) but with
TABLE 1 Effect of Inflow (a) Hypoxia on Outflow (v) pH, PO₂, PCO₂, O₂ Extraction, MVO₂, and Mean Coronary Flow

<table>
<thead>
<tr>
<th></th>
<th>PO₂ (torr)</th>
<th>pHv (units)</th>
<th>PCO₂ (torr)</th>
<th>PO₂ (torr)</th>
<th>pHv (units)</th>
<th>PCO₂ (torr)</th>
<th>O₂ Extraction (%)</th>
<th>Mean flow (ml/min per g)</th>
<th>MVO₂ (μl/min per g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>530 ± 4</td>
<td>7.42 ± 0.01</td>
<td>34 ± 1</td>
<td>119 ± 9</td>
<td>7.31 ± 0.01</td>
<td>45 ± 1</td>
<td>76.5 ± 1.8</td>
<td>2.8 ± 0.1</td>
<td>32 ± 1</td>
</tr>
<tr>
<td>5</td>
<td>461 ± 10</td>
<td>7.40 ± 0.01</td>
<td>35 ± 1</td>
<td>102 ± 18</td>
<td>7.28 ± 0.01</td>
<td>46 ± 2</td>
<td>77.9 ± 3.8</td>
<td>3.1 ± 0.2</td>
<td>31 ± 1</td>
</tr>
<tr>
<td>10</td>
<td>370 ± 5</td>
<td>7.42 ± 0.01</td>
<td>33 ± 0</td>
<td>62 ± 8</td>
<td>7.32 ± 0.01</td>
<td>44 ± 1</td>
<td>83.3 ± 2.1</td>
<td>3.7 ± 0.2</td>
<td>31 ± 2</td>
</tr>
<tr>
<td>9</td>
<td>271 ± 5</td>
<td>7.43 ± 0.01</td>
<td>32 ± 1</td>
<td>32 ± 3</td>
<td>7.32 ± 0.01</td>
<td>42 ± 1</td>
<td>87.9 ± 1.0</td>
<td>4.4 ± 0.1</td>
<td>29 ± 2</td>
</tr>
<tr>
<td>3</td>
<td>172 ± 2</td>
<td>7.44 ± 0.04</td>
<td>34 ± 4</td>
<td>19 ± 6</td>
<td>7.32 ± 0.03</td>
<td>43 ± 3</td>
<td>88.4 ± 2.3</td>
<td>4.9 ± 0.6</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>8</td>
<td>31 ± 5</td>
<td>7.43 ± 0.01</td>
<td>29 ± 2</td>
<td>10 ± 2</td>
<td>7.28 ± 0.01</td>
<td>45 ± 2</td>
<td>68.8 ± 3.6</td>
<td>5.4 ± 0.4</td>
<td>3 ± 1</td>
</tr>
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</table>

All values are means ± SEM.
* Normoxia; † moderate hypoxia (MVO₂ constant); ‡ severe hypoxia.

Higher conductance intercepts; the intercepts for 1 and 2 μM adenosine were similar (P < 0.001) but higher than control, whereas the intercept for 20 μM adenosine was higher than that for 1 and 2 μM adenosine (P < 0.05). On the other hand, the conductance responses to higher doses of adenosine (200, 2000, and 5000 μM) were not affected by the PO₂v, i.e., the slopes were statistically flat. The intercept for 20 μM adenosine was higher than the intercepts for 200, 2000, and 5000 μM adenosine. During extreme hypoxia (PO₂v = 10 ± 2; MVO₂ = 9% of normoxic control), dilation was maximal but at a lower level than observed during mild hypoxia plus adenosine.

Figure 4 depicts the volume flow in excess of the steady-state flow during normoxia (PO₂v = 119 torr) or during increased levels of hypoxia with increasing doses of adenosine. The volume overflow for lower doses of adenosine (1, 2, and 20 μM) was not affected by the PO₂v at constant MVO₂ and the slopes were similar (P < 0.01). With higher doses (200–5000 μM), volume overflow decreased concomitantly with the higher baseline flows associated with more severe hypoxia.

Effect of Occlusion Plus Hypoxia

Baseline conductance rose by 98% (5.1 ± 0.2 to 10.1 ± 0.7 ml/100 g per torr per min) with maximum hypoxia alone; peak conductance rose by 98% (5.1 ± 0.2 to 10.2 ± 0.8 ml/100 g per torr per min) with a 30-second occlusion during normoxia (Fig. 5). The duration of occlusion (0–30 seconds) was related linearly to peak conductance (r = 0.73; P < 0.01). Baseline conductance also was related inversely to the severity of hypoxia between 119 and 33 torr PO₂ (r = −0.42; P < 0.01). I found no difference between the slopes of baseline conductance and peak conductance following a 5-second occlusion; i.e., they varied proportionately as a function of PO₂v (P < 0.01). However, the slope of peak conductance after occlusion had a higher intercept. Peak conductance (or peak diastolic flow) responses to occlusion of 15 and 30 seconds, on the other
hand, were not statistically affected by the reduction in PO$_2$V, although Figure 5 shows a trend. Thus, the effect of a 5-second occlusion during hypoxia was merely additive to the effect of hypoxia alone; i.e., the slopes were similar, but the conductance intercept was greater with a 5-second occlusion than without occlusion; 15- and 30-second occlusions resulted in similar but insignificant slopes.

Both the duration (27 ± 2 seconds, 40 ± 3 seconds, 60 ± 5 seconds) and the volume of reactive hyperemia (volume recovered; 0.41 ± 0.03, 0.72 ± 0.09, 1.16 ± 0.10 ml/g) for 5-, 15-, and 30-second occlusions, respectively, were unchanged by hypoxia between 119 and 33 torr PO$_2$V (not shown). Since baseline flow increased with hypoxia, the volume flow deficit incurred during occlusion (preocclusion flow times occlusion time) concomitantly increased. With 5-second occlusions, the volume recovered (time integrated flow in excess of baseline flow during period of overflow) approximately equalled the volume deficit of occlusion with all levels of moderate hypoxia (Fig. 6). The percentage of repayment (100 times volume recovered/volume deficit) was therefore 100 (±7)%.

As shown, for longer occlusions and moderate hypoxia, the deficit usually exceeded that recovered. For 15-second occlusions, the percentage of repayment was 113 ± 10, 81 ± 6.4, 89 ± 19, and 69 ± 12 with PO$_2$V levels between 119 and 33 torr; for 30-second occlusions, the percentage of repayment was 90 ± 7, 83 ± 12, 80 ± 12, and 58 ± 8. With maximal flow during severe hypoxia (PO$_2$V = 10 torr), reactive hyperemia could not be observed following any occlusion time and the percentage of repayment was nil.

**Discussion**

This study was designed to test whether the flow increase following exogenous adenosine injection or the reactive hyperemia following occlusion is augmented or potentiated during moderate hypoxia. I observed that, whereas adenosine injection and occlusion consistently increase peak flow above the level observed during normoxia, the increment of flow increase with either maneuver becomes progressively smaller the more severe the hypoxia. Statistical analyses showed that, at constant MVO$_2$, low doses of adenosine (1, 2, and 20 µM) or a 5-second occlusion produce an increase in flow which is merely additive to the increase in baseline flow resulting from hypoxia alone; higher doses of adenosine (200 to 5000 µM) or longer occlusions (15 and 30 seconds) have a diminishing effect on elevating peak flow as baseline flow rises to higher levels when perfusate PO$_2$ is reduced. The volume of flow above baseline due to adenosine injection and oc-
CORONARY FLOW WITH LOW O₂, ADENOSINE, AND OCCLUSION/Stowe 397

clusion, although greatest with increasing doses or occlusion time, was unaffected during moderate hypoxia but was decreased with severe hypoxia. The volume of reactive hyperemic flow also was unaffected by the level of moderate hypoxia but, since baseline flow and thus also the flow deficit increased with hypoxia, the flow repayment necessarily decreased and was dependent on the severity of hypoxia and on the length of occlusion. It would appear from this study that, as hypoxia-induced vasodilation increases, the capability of vasodilators, whether endogenous or exogenous, to promote further flow increment is diminished. Alternate responses to adenosine injection or occlusion might have been a potentiated hyperemic response, at least up to the level of maximal vasodilation, or no added rise in flow during hypoxia.

I have used the isolated, paced, nonworking heart for these studies because it offered the advantage of easily measuring coronary flow relatively unaffected by preload and afterload factors and by nerve stimulation. Metabolism is aerobic and is similar to that in vivo (Bünger et al., 1975). In the range of mild hypoxia where MVO₂ remains constant, peak left ventricular pressure, measured using a latex balloon, and dP/dt also were constant (unpublished observation). Since nearly identical results were observed in similar preparations (Arnold et al., 1968; Rubio et al., 1974), compressive factors on the coronary vessels probably were stable during mild hypoxia. A potential disadvantage of this preparation is that it may not possess a "coronary reserve" as large as that of the intact, blood-perfused heart. Maximal flow was only approximately two times the resting value, whereas in dogs it may be quadrupled. Nevertheless, I was able to conduct dose-response curves for both hypoxia and adenosine or reactive hyperemia while MVO₂ remained constant. The decrease in arterial O₂ content with blood-free perfusate may be expected to account for increased baseline coronary flow, but this effect can be buffered by the decreased left ventricular pressure development and by the lack of external work performed by the Langendorff hearts. During mild, graded hypoxia, the hearts maintained MVO₂ by increasing both coronary flow and the percentage of O₂ extracted. During severe hypoxia, flow was at or near maximum and MVO₂ (and work) was depressed. Interpretation of the interaction of various possible vasodilators under this condition is questionable at best; consequently statistical analysis of interactive effects was limited to data for which MVO₂ was constant.

Combined Changes in Metabolic Factors Affecting Coronary Flow

In recent years, investigators have suggested that metabolic factors might interact to elicit vasomotion (Haddy and Scott, 1968; Haddy and Scott, 1975). There are, however, few reports of the possible interaction of hypoxia and adenosine, and of hypoxia and occlusion on coronary flow even though hypoxia and tissue release of adenosine have long been thought to be intimately related to coronary blood flow regulation (Berne, 1964). Gellai et al. (1973) found that in strips of rabbit coronary arteries contracted by acetylcholine, relaxation by adenosine was progressively greater with a drop in the bath PO₂; without adenosine, low bath PO₂ had no effect on strip tension. Moir and Jones (1973) used intact dogs breathing 5% O₂ and observed that the coronary vascular response to adenosine was enhanced when compared with breathing 100% O₂; intermediate fractions of O₂ produced responses varying from attenuation to enhancement. Lammerant and Becsei (1972) infused adenosine into the right atrium of dog hearts and found it lowered coronary resistance to 25% of control; coupling infusion of adenosine with breathing 100% O₂ raised coronary resistance slightly to 37% of control. Comparison of the results of these earlier studies with mine is difficult because of differences in the models and methods used to test this hypothesis.

More recently, the combined effects of hypoxia and hypercapnia, adenosine and potassium ion, and adenosine and acidosis on the coronary circulation have been reported. Ventilating dogs with CO₂ to produce hypercapnic acidosis has been shown to increase the coronary dilator response to a single intracoronary dose of adenosine (Raberger et al., 1975). In the isolated perfused guinea pig heart, increasing the percentage of CO₂ in the perfusate decreased perfusate pH and reduced the concentration at which adenosine produced a response; also the increased flow with a drop in pH was greater in the presence of adenosine (Merrill et al., 1978). Simultaneous addition of adenosine (0.1 to 100 μM) and K⁺ (bath 2 mM to 4 mM) to acetylcholine-stimulated cat coronary vascular strips produced greater dilation than either potassium ion or adenosine alone (Foley et al., 1979). Acidosis (pH 7.0) and hypoxia (20% O₂) increased coronary flow in isolated, perfused guinea pig hearts from 2.7 and 3.4 individually to 4.0 ml/min per g when acidosis and hypoxia were combined; control flow (pH 7.4; 95% O₂) was 2.4 ml/min per g (Degenering, 1976). In the same study, an intracoronary injection of 0.5 ml of 5 μM adenosine effected no flow change from control (pH 7.4) with acidosis (pH 7.0) but caused a reduction with alkalosis (pH 7.8) and 95% O₂ in the perfusate. Case et al. (1978) measured a broad range of coronary sinus PO₂ and PCO₂ with separate changes in arterial O₂ and CO₂ fractions and concluded that the sensitivity of coronary vascular resistance to O₂ change was approximately twice that due to CO₂ change. The above studies show that, with increased hydrogen ion or potassium ion concentration in the presence of adenosine or hypoxia in the presence of acidosis, vasodilation is accentuated; however, it is not evident whether the
vascular effects were merely additive or truly potentiated.

Comparison of Responses to Adenosine and Occlusion during Hypoxia

Results of several studies suggest that reactive hyperemia is closely dependent on either the O2 and energy debt developed during occlusion or the associated accumulation of vasoactive metabolites. However, the volume of reactive hyperemia may not be proportional to the flow rate before occlusion. Coffman and Gregg (1961) noted that, for a constant duration of occlusion, increasing baseline flow caused a less than proportional increase in the volume of reactive hyperemia. When O2 demand and consequently coronary blood flow were increased by paired ventricular pacing before occlusion, Bache et al. (1973) found that the volume of reactive hyperemia increased, but the percentage of repayment of flow was unchanged from control; moreover, during adenosine infusion which elevated baseline flow but not MVO2, the volume of reactive hyperemia was no different from the response without adenosine.

The data presented here show that, during a constant MVO2, increased levels of hypoxia affected neither the duration nor volume of reactive hyperemia following up to a 30-second occlusion; however, the rise in peak flow above preocclusion control was reduced, and the net flow debt (occlusion flow deficit minus flow repayment) was decreased as the preocclusion flow increased with hypoxia. These data are consistent with those of previous studies. The hearts responded to the decreased inflow O2 content both by increasing coronary flow and by increasing the O2 extracted relative to arterial levels; consequently, MVO2 was maintained. The net O2 debt incurred with a given occlusion could be the same at any level of hypoxia as long as MVO2 is unchanged. Moreover, since the volume of reactive hyperemia was found not to depend on the preocclusion flow, it could be expected to vary directly with a change in MVO2. For submaximal occlusions of 5 seconds, there was 100% repayment of the flow deficit throughout moderate hypoxia (MVO2 constant); when PO2 dropped further, volume flow dropped along with a drop in MVO2. For occlusions of 15 and 30 seconds, there were progressively lesser net repayments because the flow deficit progressively increased as baseline flow rose with hypoxia. Thus, it appears that once the O2 (or energy) debt is repaid and accumulated metabolites removed, flow returns to a level which is actually independent of the level of control flow, i.e., the reactive hyperemic response is related only to the O2 (or energy) debt or resulting metabolic excess incurred during occlusion.

Several laboratories have reported that, concomitant with an increase in coronary flow, adenosine is released into the effluent of the isolated perfused guinea pig heart during severe hypoxia (PO2 < 30 torr) (Rubio et al., 1974; Schrader et al., 1976; Schrader et al., 1977a). 14C-labeled adenosine is released in the same preparation during reactive hyperemia following occlusions of 15, 30, and 60 seconds (Schrader et al., 1977a). Olsson et al. (1978) compared canine myocardial tissue adenosine levels during reactive hyperemia following 5- to 15-second occlusions with tissue levels obtained with flow increases during step infusions of adenosine. They found that endogenous and exogenous adenosine levels decreased exponentially during reactive hyperemia and thus suggested adenosine could account for the flow response. They also calculated that, for one-half the maximal conductance, the tissue adenosine concentration with adenosine infusion was one-half that during reactive hyperemia, and that the log adenosine slope for reactive hyperemia was steeper than that for adenosine infusion. This finding might be due to enhancement of the vasodilatory effect of adenosine during reactive hyperemia or due to additive vasodilatory effects of oxygen lack or of other metabolites which accumulate during occlusion but not during adenosine injection. There is evidence for the existence of specific adenosine receptors (Olsson et al., 1976; Schrader et al., 1977b). Moreover, using the guinea pig heart preparation, Wiedmeier et al. (1978) suggest that there may be two different adenosine receptors, one responsive to exogenous adenosine (blocked competitively and rapidly by theophylline) and another responsive to endogenous adenosine (blocked slowly and irreversibly by theophylline). Burstock (1980) considers that there are receptors most sensitive to adenosine and AMP (P1 purinoceptors) and receptors most sensitive to ATP and ADP (P2 purinoceptors). These and many other related studies showing an increased myocardial tissue concentration of adenosine during hypoxia and reactive hyperemia strongly suggest that the coronary resistance vessels are exposed to high concentrations of adenosine during these stresses.

Based on my findings and those of others, I offer the following interpretation of the effects of adenosine and occlusion on vasoactivity during hypoxia: (1) As the oxygen supply to the myocardium is decreased stepwise, tissue O2 (and PO2) decreases, resulting in an enhanced rate of release of endogenous adenosine or other purines and an increase in coronary flow. Tissue ATP and total energy charge remain stable because MVO2 is maintained by the increased flow and higher O2 extraction. (2) Assuming that the number of adenosine receptors occupied is directly proportional to the level of oxygen debt (and/or increased release of adenosine) and that the total number of adenosine receptors remains constant and equally accessible, then submaximal doses of adenosine, given exogenously, would produce only an additive vasodilatory response; i.e., the exogenous adenosine must compete...
with the endogenously released adenosine for receptor sites. With maximal dilation due to high doses of adenosine, severe hypoxia, or longer occlusion, all adenosine receptors would be occupied. (3) Similarly, the reactive hyperemic response (flow repayment), although greater as the duration of occlusion increases due to longer transient hypoxia and increased accumulation of metabolites, would be unaffected by the level of hypoxia (if $\text{MVO}_2$ remains stable). Again, the baseline flow during hypoxia might be closely related to the ratio of adenosine release/uptake and occupation of its receptor sites; the vasodilatory response to an increased rate of release of adenosine, due to hypoxia and release of other metabolites following occlusion, would necessarily be limited by the number of available receptors remaining. (4) Furthermore, since the dose response to adenosine is not potentiated but rather additive with low doses and attenuated with high doses during mild hypoxia, hypoxic vasodilatation per se is most likely effected through release of endogenous adenosine.

Although I have used occupation and eventual saturation of adenosine receptors to explain my results, one could consider that accumulation of other metabolites during hypoxia, e.g., ATP, also might compliment the vascular response to administration of adenosine by occupying non-adenosine receptors which also leads to dilation. Alternatively, these other metabolites could affect the way adenosine occupies its own receptors.

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D F Stowe

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